region and so allows the helicase and polymerase to bind and begin unwinding and replication, respecively. After travelling all around the plasmid probably as a single protein complex, the proteins then reach the newly synthesised origin of replication, which provides the signal for termination. RepD then does a series of strand exchanges to close the two plasmid circles.
We are using a combination of measurements with whole plasmids and oligonucleotide models to elucidate the series of events at each stage of the replication. In particular, by following individual processes in real time, we are able to describe the order of biochemical steps that enable this process to occur.

363-Pos Board B143
Cooperative Activity of SARS Coronavirus Nsp13 Helicase Characterized by Single Molecule FRET
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SARS was epidemic in 2003 worldwide. SARS-CoV helicase plays critical roles in viral replication, and has been proposed to be a potential candidate for anti-SARS therapy. We use single molecule fluorescence resonance energy transfer to examine the unwinding and rewinding mechanism of nsP13 helicase on partial DNA duplexes as a function of protein, ATP concentration, and tail length. Our results reveal that the tail length of the substrates determines the total amount of DNA unwound by increasing the number of proteins loaded. In contrast, unwinding rate and step size increase as a function of the protein and ATP concentration for the partial duplex with a long tail (45nts long), but independent of protein concentration for the short tail (30nts long). We also observed a repetitive unwinding displaying multiple rounds of re-unwinding and re-zipping events where re-unwinding becomes favorable at higher protein concentration. We also found that the relative extent of constitutive unwinding and repetitive fluctuation is defined by the modality of DNA-Protein complex in the presence or absence of ATP concentration. The ratio between them determines the processivity of the cooperative helicases in tandem. In general, our results identify the important cellular parameters, governing the cooperative unwinding and repetitive rewinding behavior of helicase. This is a new attempt to understand the complicate behavior of unwinding motor cohorts at the single molecule resolution.

364-Pos Board B144
Measuring the Kinetics of Restriction Endonucleases with Single Molecule Resolution
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We have developed a simple assay for observing the cleavage of DNAs with single molecule sensitivity. DNAs are attached to a surface at one end using a digoxigenin-antibody link and to a magnetic micro bead at the other end via a biotin-streptavidin link. The DNAs are stretched by applying fluid drag and magnetic forces. The exact time of cleavage of individual DNAs is recorded with video microscopy by observing the time of disappearance of each bead. We are using our technique to measure the kinetics of two type II restriction endonucleases, EcoRI and NdeI. With our kinetic data, we hope to elucidate the target site search mechanisms of these enzymes.

365-Pos Board B145
Dynamic Control of Processivity during DNA Degradation by a Ring-Shaped Nuclease
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DNA exo-nucleases catalyze numerous essential biological processes such as DNA replication, recombination, and repair. λ exo-nuclease (λ exo) is composed of three active sites and forms a ring structure for processive degradation. The detail molecular basis governing the allostery of trimer has not been well understood yet. Here we used single molecule fluorescence resonance energy transfer (FRET) to examine how the three enzymatic sites of λ exo are coordinated. We find that only one of three active sites is utilized and the ring of λ exo is rotated along DNA helix during degradation. We further examine how the previous motion of λ exo-nuclease influences on the following enzymatic activity, and found that the continuous cleavage activity guides the enzyme to competently position, making it tilted around 45° to the DNA helical axis. This coordinated comprehensive motion is required for efficient and processive degradation, suggesting a hierarchy nature for processivity. We also find that the tendency of backtracking on ssDNA increases when the degradation rate slows down.

366-Pos Board B146
The Effect of Single-Stranded DNA Binding Protein RPA2 on XPD Helicase Processivity
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FacXPD helicase is the archaeal homolog of yeast Rad3 and human xeroderma pigmentosum group D protein (XPD) from the organism Ferroplasma acidarmanus. This enzyme serves as a model for understanding the molecular mechanism of human superfamily 2B helicase XPD involved in transcription initiation and nucleotide excision repair. Previous work has shown that the unwinding of double-stranded DNA by FacXPD is regulated by the single-stranded DNA binding protein FacRPA2. However, the mechanism by which this occurs is unknown. Here, we present a single molecule study of this regulation using optical traps. We show that XPD is a weak helicase as a monomer, only able to unwind short distances (~12 bp) under tension applied by the optical traps, with a strong dependence on DNA sequence. In the presence of RPA2, however, XPD monomers are able to unwind more processively (>90 bp). Using a combination of optical traps and a laminar flow cell, we performed experiments in which we loaded XPD, RPA2, and provide ATP in a controlled sequence. We used this approach to distinguish between different potential mechanisms of regulation. Our data disfavor mechanisms by which RPA2 regulates XPD activity by sequestering ssDNA. Instead our results suggest that RPA2 forms a complex with XPD, activating it for processive unwinding. RPA2 can also bind ahead of the traveling helicase and destabilize the duplex junction.

367-Pos Board B147
The Role of DNA Shape in Nucleosome Formation and Positioning
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Nucleosomes provide the basic unit of compaction in eukaryotic genomes and the mechanisms that dictate their position along a DNA sequence are of central importance to epigenetics. In this work, we use molecular models of DNA and proteins to explore various aspects of nucleosome positioning. First, we show how the affinity of DNA for histones is encoded in its sequence-dependent shape, including subtle deviations from ideal straight B-DNA and local variations in minor groove width. Using high-precision simulations of the free energy of nucleosome complexes, we also demonstrate that, depending on DNA's intrinsic curvature, histone binding can be dominated by bending interactions or electrostatic interactions. Secondly, we examine the sequence-dependent mechanism by which DNA slides to its optimal position around the histone proteins. More generally, the results presented here explain how sequence, manifested as the shape of the DNA molecule, dominates molecular recognition in the problem of nucleosome formation.

368-Pos Board B148
Polymorphism of Histone Tail Interactions in Nucleosome
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Histone tails protruding from nucleosome core are known to play important role in gene expression regulation. They affect nucleosome dynamics and remodeling, chromatin compaction, transcription initiation and elongation and serve as targets for multiple posttranslational modifications, known as histone-code. The disordered nature of histone tails makes their structural characterization elusive. To address this question for the first time we perform extensive microsecond molecular dynamics simulations of nucleosome including linker DNA in explicit solvent in order to analyze the evolution of histone tails' conformation and their interaction patterns with nucleosome core and linker DNA. We show that histone tails readily bind to nucleosomal DNA and become kinetically trapped in a certain number of distinct conformational states at microsecond timescale. Certain bound states of histone tails are associated with secondary structure formation, rearrangement of DNA conformation and protein DNA-interactions in the nucleosome core as well as neutralization of linker DNA. The implications of our findings for nucleosome dynamics, chromatin fiber compaction and histone code functioning are discussed.